CLAIMS

1. An enzymatic process to obtain 4-O-ß-D-galactopyranosyl-D-xylose that comprises:

a first step of preparation of a first reaction mixture of

2-20% by weight of D-xylose

0-5-5% by weight of a ß-D-galactopyranoside substrate

75-97.5% by weight of a reaction medium that comprises buffered water at a pH between 5.0 and 9.0;

adding 10 to 1,000 units of a ß-D-galactosidase enzyme, per gram of ß-D-galactopyranoside, to the first reaction mixture; and obtaining a second reaction mixture

a second step wherein the second reaction mixture is subjected to a reaction at a temperature comprised between a temperature higher than the freezing point of the second reaction mixture and 45ºC, for 2 to 48 hours, in order to form disaccharides in the second reaction mixture;

a third step wherein the reaction is stopped when the disaccharides have been formed in the desired amount, by means of a treatment chosen between deactivation of ß-D-galactosidase by freezing the second reaction mixture at a temperature between 20ºC and -170ºC, deactivation of ß-D-galactosidase by heating the second reaction mixture at a temperature between 95 and 110ºC, and separation of ß-D-galactosidase from the second reaction mixture by ultrafiltration; obtaining a third reaction mixture;

a fourth step wherein an aglyconic fragment of the ß-D-galactopyranoside substrate used in the first step is separated from the third reaction mixture by extraction or filtration; obtaining a fourth reaction mixture;

a fifth step comprising isolation of fractions that contain 4-0-ß-D-galactopyranosyl-D-xylose, **characteriz d** in that, the fifth step is selected between addition of

celite to the fourth reaction mixture, followed by solid-liquid extraction with a solvent and elution with a first eluent in a column; and directly adding active carbon to the fourth reaction mixture followed by filtration and elution with a second eluent, and in that, in a sixth step, the fractions that contain 4-O-ß-D-galactopyranosyl-D-xylose, are crystallized in a crystallization mixture selected among mixtures of acetone/methanol in a ratio between 5/1 to 20/1 and mixtures of acetone/water in a ratio between 5/1 to 20/1.

2. Process according to claim 1, characterized in that the fourth reaction mixture is concentrated before being subjected to elution in the column.

3. Process according to claim 1, characterized in that the mixture of acetone/methanol has a ratio of 10/1.

4. Process according to claim 1, characterized in that the mixture of acetone/water has a ratio of 10/1.

5. Process according to claim 1, characterized in that the first eluent is a mixture of water/isopropanol that contains 1 to 10% (v/v) of isopropanol.

6. Process according to claim 1, characterized in that the mixture of water/isopropanol contains 2% (v/v) of isopropanol.

7. Process according to claim 1, characterized in that the fifth step consists of adding celite to the fourth reaction mixture and concentrating to dryness, followed by solid-liquid extraction with an organic solvent in a Soxhlet extractor that has a cartridge made out of a material compatible with said solvent, and eluting with a first eluent in a column selected among

filtration columns with cross-linked dextrane polymer fillers, filtration columns with acrylamide polymer fillers, filtration columns of active carbon or active carbon-celite columns.

8. Process according to claim 7, characterized in that the solvent is ethyl acetate.

9. Process according to claim 7, characterized in that the solvent is used in an amount comprised between 10 ml and 25 ml per gram of initial xylose.

10. Process according to claim 7, characterized in that the celite is used in an amount comprised between 1 g and 2 g per gram of initial xylose.

11. Process according to claim 7, characterized in that the column is of active carbon-celite wherein the carbon is deactivated by adding 35% hydrochloric acid.

12. Process according to claim 11, characterized in that the celite is used in an amount comprised between 0.5 g and 2 g of celite per gram of initial xylose.

13. Process according to claim 11, characterized in that the active carbon is used in an amount comprised between 0.5 g and 2 g of active carbon per gram of initial xylose.

14. Process according to claim 7, characterized in that said first eluent is used in an amount comprised between 5 ml and 25 ml per gram of initial xylose.

15. Process according to claim 11, characterized in that the hydrochloric acid is used in an amount comprised between 0.5 ml and 1.5 ml per gram of initial xylose.

16. Process according to claim 1, characterized in that in the fifth step, the fourth reaction mixture is subjected to direct addition of at least a second eluent on the active carbon wherein the 4-O-ß-D-galactopyranosyl-D-xylose is adsorbed on the active carbon and the second eluent is water followed by diluted isopropanol with a growing proportion in volume of isopropanol in successive steps.

17. Process according to claim 16, characterized in that the proportion in volume of isopropanol is comprised between 1% and 3% in a first step, between 3% and 5% in a second step and between 5% and 7% in a third step.

18. Process according to claim 16, characterized in that the active carbon is used in an amount comprised between 2 g and 4 g of active carbon per gram of initial xylose.

19. Process according to claim 16, characterized in that the second eluent is used in a total amount comprised between 30 ml and 50 ml of second eluent per gram of initial xylose.

20. Process according to claim 1, characterized in that the reaction is stopped by cooling the second reaction mixture at 0°C.

21. Process according to claim 1, characterized in that the fourth reaction mixture is obtained by separating the aglyconic fragment from the β-D-galactopyranoside substrate by means of filtration.

22. Process according to claim 1, characterized in that the proportion of D-xylose in the second reaction

mixture is 7.5% by weight.

23. Process according to claim 1, characterized in that the proportion of β-D-galactopyranoside in the second reaction mixture is 1.5% by weight.

24. Process according to claim 1, characterized in that 20 units of β-D-galactosidase per gram of β-D-galactopyranoside are added.

25. Process according to claim 1, characterized in that the reaction medium also comprises at least a cosolvent medium selected among dimethylsulfoxide, dimethylformamide, dioxane and mixtures thereof.

26. Process according to claim 25, characterized in that the reaction medium comprises 20% by weight of the cosolvent medium.

27. Process according to claim 1, characterized in that the reaction is carried out at a constant temperature.

28. Process according to claim 1, characterized in that the reaction temperature is from -5°C to 40°C.

29. Process according to claim 1, characterized in that the reaction temperature is higher than the freezing temperature of the second mixture and lower than 0°C.

30. Process according to claim 1, characterized in that the reaction temperature is -5°C.

31. Process according to claim 1, characterized in that the reaction temperature is room temperature.

32. Process according to claim 1, characterized in that the reaction medium is buffered to a pH of 7.

33. Process according to claim 1, characterized in that, in the third step, the reaction is stopped by freezing the second reaction mixture at a temperature of -78°C.

34. Process according to claim 1, characterized in that, in the third step, the reaction is stopped by heating the second reaction mixture up to a temperature of 100°C.

35. Process according to claim 1, characterized in that, in the third step, the reaction is stopped by separating the β-D-galactosidase by ultrafiltration.

36. Process according to claim 1, characterized in that the β-D-galactopiranoside substrate is selected between o-nitrophenyl β-D-galactopiranoside and lactose.

37. Process according to claim 1, characterized in that the β-D-galactosidase enzyme is *E. coli* β-D-galactosidase.

38. Process according to claim 1, characterized in that the β-D-galactosidase enzyme is *Kluyveramyces lactis* β-D-galactosidase.

39. A 4-O-ß-D-galactopyranosyl-D-xylose characterized in that it has been obtained by means of the process defined in claim 1.

40. A composition for *in vivo* evaluation of intestinal lactase in humans, characterized in that it comprises a 4-O-ß-D-galactopyranosyl-D-xylose obtained

by means of the process defined in claim 1.

41. A solution for the *in vivo* evaluation of intestinal lactase in humans, characterized in that it comprises a solution selected between aqueous solutions and saline solutions of a 4-0-ß-D-galactopyranosyl-D-xylose obtained by means of the process defined in claim 1.

42. Use of 4-0-ß-D-galactopyranosyl-D-xylose prepared according to claim 1, in the preparation of a composition for *in vivo* evaluation of intestinal lactase in humans.

43. Use of 4-0-ß-D-galactopyranosyl-D-xylose prepared according to claim 1, in the preparation of a solution selected between saline solutions and aqueous solutions for *in vivo* evaluation of intestinal lactase in humans.

44. Use according to claim 42, characterized in that the 4-O-ß-D-galactopyranosyl-D-xylose is combined with pharmaceutically acceptable amounts of at least one additive selected from among stabilizers, protecting agents, flavoring agents, lactose, gelling agents, fluidizing agents and preservatives.